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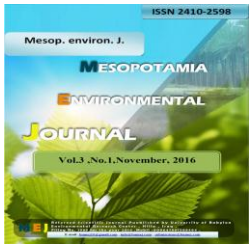

Comparison between SSR and RFLP techniques for genetic diversity of Iraqi date palm (*Phoenix dactylifera* L.)

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Abstract

This study used 5 microsatellites to determinate genetic diversity among sixty five date palm cultivars, profile of all of these by separated Simple Sequence Repeat SSR-PCR products on the gel electrophoresis either one band (homozygous) or two bands (heterozygous) were revealed for each primer based on the types of microsatellite loci. And Restriction Fragment Length Polymorphism RFLP- PCR products separated on the gel acrylamide. The results in this study revealed that the total number of allele with 5 microsatellites was 95 alleles, average of amplified bands was 66 bands and the percentage of polymorphic bands was 77%. The results of matrix revealed the similarity between date palm cultivars with SSR primers represented by the highest distance was (1.2371) between (Smeasmi) and (Sabb Drrah), that means no similarity with them cultivars, while the lowest degree of distance was (zero), which represented no distance between cultivars were, (Shwethi) and (Maktom), that means presence of similarity between them. Genetic relationships among twenty four date palm cultivars estimated by SSR primers from unweighted pair-group method (UPGMA) dendrogram. Another wise this study used universal primers internal transcribed 2. spacer ITS Forward and ITS Reverse, The analysis was performed to reveal varieties fingerprinting with the Sultana, Sukkary and Maktom cultivars that mean number of fingerprinting was 3.The results of RFLP in this study revealed that the total number of amplified bands were 48 bands and the percentage of polymorphic bands were 100%.

Introduction

Date palm (*Phoenix dactylifera* L.*)* is the major fruit crop of arid climate regions. It (2n=2x=36) was considered of great socioeconomic importance in the rabian region[1]. The number of known date palm cultivars that are distributed all over the world are 5000 of which 600 are found in Iraq. Before 1991, Iraq was the largest producer of dates in the world[2] and had the largest date forest in the world[3]. However, during the Gulf and Iran- Iraq wars, Iraqi number of date palm trees was destroyed.Wars and sanctions imposed on Iraq have negatively affected both the production and natural genetic diversity of the crop in Iraq and inhibited the much- needed impetus to rebuild the date palm industry[4]. The unique characteristics of date palm can be truly called "tree of life" and is considered as one of the most ancient plant. The rich fruit plays an important role in the nutrition of human population, and also several products are made that generate employment and thus influence socioeconomic aspect of people. Therefore, it is widely acknowledged sustainability value in social, economic and ecological terms. Moreover, this crop has great potential as a source of renewable energy, by producing bio-fuel since its fruits high in carbohydrates 44-88% total sugars[5]. In spite of the date palm is one of the

oldest 3cultivated fruit trees, but there are a few genetic resources for improving the productivity and development of the dioecious date palm[6]. DNA-based markers and its traits in date palm progeny segregation could be used for selection instead of morphological traits. DNA fingerprinting, also known as DNA typing or genetic fingerprinting, uses for identifying individuals by the particular of their DNA. There are many molecular markers applied to identify date palm cultivars, for understanding and analyze the genetic relationships and genetic diversity among date palm varieties. Microsatellites or Simple Sequence Repeats (SSRs) are di-,tri-or tetra- nucleotide repeats reveal as abundant, dispersed throughout the genome and move highly polymorphic than other genetic markers in eukaryotic genome. The SSR markers are used to analyze the genetic diversity among date palm cultivars to develop a DNA fingerprints[7]. Restriction Fragment Length Polymorphisms (RFLPs) technique used with the variation in the length of DNA fragments produced by a specific restriction endonuclease from genomic DNAs[8]. Few studies have been used RFLPs on date palm because of the relatively large amounts of DNA required to obtain an adequate sample, and the very high cost due to labor and reagent expenses[9]. The main aim of the present study is to investigate the suitability of the RFLP and SSR markers to distinguish some date palm varieties and to detect genetic diversity in natural field populations.

Materials and Methods

Plant Materials

Fifty six date palm females' cultivars and nine males as date superior pollinators collected from Hillah city in Iraq. The young whit leaves collected, upthe palm nearby heart of the tree from all genders, which represented number of cultivars per species from different locals. Table 1 illustrated these details.

Extraction DNA

Leaves of date palm cultivars for all species used in this study, which were collected from Hilla city. Leaves (200mg) were grounded to a powder using liquid nitrogen and placed in the microfuge tubes then DNA were extracted by using Mini Kit (Geneaid Biotech. Ltd; Taiwan company), for yield purifying total DNA including genomic DNA, chloroplast and mitochondrial DNA, from plant tissue according to manufacturer manual.

Primers:

All primers used in this study were provided by Bioneer Corporation in deionized Distilled water to obtain 100 Pmol/ml as a final concentration (stock solutions). The concentration 10 pmole/ml of primers was prepared from stock solutions. five primers of SSR markers were used in this study. Listed in table 2 and mentioned their names and nucleotide sequences of each primer.

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Table 1: Details of sixty five date palm cultivars were grown in Hilla city.

No.	Cultivar	No. of Cultivar	Code	Gender
1	Bream	3	A	Female
2	Tebarzal	3	B	Female
3	Sabb Drrah	3	C	Female
4	Hamrawi	3	D	Female
5	Brban	3	E	Female
6	Ashrasi	3	F	Female
7	Zahdi	3	G	Female
8	Sultana	3	H	Female
9	Khadrawi	3	I	Female
10	Sukkary	3	J	Female
11	Khestawi	3	K	Female
12	Usta Umran	3	L	Female
13	Guntar	3	M	Female
14	Maktom	3	N	Female
15	Nersi	3	O	Female
16	Maddany	3	P	Female
17	Barhi	2	Q	Female
18	Chipchab	2	R	Female
19	Najdi	2	S	Female
20	Fom Alrman	1	T	Female
21	Shwethi	1	U	Female
22	Greatli	3	V	Male
23	Ghanami Ahmer	3	W	Male
24	Smeasmi	3	X	Male

Tabel 2: List of SSR primers used in this study.

No.	Primer name	Forward primer(5′-3′)	Reverse primer(3′-5′)
1	mPdCIR010	ACCCCGGACGTGAGGTG	CGTCGATCTCCTCCTTTGTCTC
2	mPdCIR015	AGCTGGCTCCTCCCTTCTTA	GCTCGGTTGGACTTGTTCT
3	mPdCIR016	AGCGGGAAATGAAAAGGTAT	ATGAAAACGTGCCAAATGTC
4	PDCAT4	TAACGAGTCCACACAC	CTGGGTAAAGCTTATAAG
5	PDCAT5	GGCCCGTCCTTGGATTAGAG	CTACGTTGTCCCGTCAATTGG

The references of (1, 2 and3) are [10], and (4 and 5) are [11].

The Internal Transcribed Spacer (ITS) primer used in this study and twenty four date palm cultivars amplified by PCR to yield one band each of date palm cultivars. The sequences of primers were

Forword: ITS1:5́-TCCGTAGGTGAACCTGCGG-3́

Reverse: ITS4: 5́- TCCTCCGCTTATTGATATGC-3́

DNA Molecular Size of Markers

Amplicon size was estimated using 100-bp DNA stander (ladder), corporation viogene. USA. Used for ISSR analysis, which description a convenient for sizing linear double- stranded DNA fragments from 100-bp to 3-Kbp. They ready- to-use DNA ladder involved of 12 double- stranded, blunt-end fragments with sizes of 3000, 2000, 1500, 1000, 800, 700, 600, 500, 400, 300, 200, and 100 base pairs.

Reaction Mixture (Master Mix)

AccuPower- PCR PreMix. Bioneer Corporation Taiwan is the convenient to perform DNA amplification. Which have description, 0.2 ml thin-wall 8-strip tubes with attached cap/96 tubes, each tube contents the component as a fallowing in table 3.

Table 3: PCR reaction mixture components.

Component Size (20µl reaction)	Reaction
Taq. DNA polymerase	1U
Each: dNTP (dATP, dCTP, dGTP, dTTA)	250 mM
Tries-HCl (pH9.0)	10mM
KCl	30mM
$MgCl_2$	1.5mM
Stabilizer and tracking dye	5 µM

Agarose Gel Electrophoresis Analysis

Gel electrophoresis methods were done according to Sambrook and Russel (2001). Agarose was made in 1% by dissolving 1g of agarose in 10ml of 10x TBE buffer and the volume was completed to 100 of distilled water.

PCR Amplification

The amplification have been used the experimental protocol of AccuPower® PCR PreMix, as following: 2µ template DNA and 2µ primer (10 pmole/1µ, 1µ forward and 1µ revers),was added to the AccuPower® PCR PreMix tube. Sterilized deionized distilled water was added to AccuPower® PCR PreMix tubes to yield the final volume of 20µl. All samples were amplified individually by using PCR apparatus and corresponding annealing temperatures. The amplification of SSR-primers have been sued the same experimental. Initial denaturation was 95ºC for 5 mints followed by 35 cycles to the 65 sample at 95ºC for 1 mint, annealing temperature was 52 ºC for 1 mint,extension temperature at 72 ºC for 1 mint, and a final extension step at 72 ºC for 7 mints. PCR products were separated by electrophoresis on 1.4% Agarose gels. Gel was visualized and imaged by U.V transilluminator[12]. Amplicons size products were estimated using 100-bp DNA ladder 100- 3000bp. The amplification of ITS- primer have been used Initial denaturation was 94ºC for 5 mints followed by 2 cycles for 65 sample,annealing temperature 59 ºC,extension 72ºC for 1mint,and by 30 cycles at 94Cº for 30 second, annealing temperature 65ºC at 45 second, extantion 72ºC at 1 mints, and a final extension step at 72Cº for 5 mints. Samples after amplified DNA were separated by electrophoresis Poly Acrylamide (1.30 hr. 90vo. Yielded fine PCR products. The results of PCR products visualized and imaged by U.V. transilluminator[12]. Amplicons size products were estimated using 100-3000 bp Ladder. Stock solution prepared 30% W/V of 29:1 of acrylamide-bisacrylamide by weighing 29gm of acrylamide and1gm of bisacrylamide then dissolved in distill water and complete the volume to 100ml. Prepared 40% W/V of 29:1 by weighing 38.66 gm of acrylamide and 1.33gm of bisacrylamide then dissolved in distill water and complete the volume to 100ml. While dissolving the acrylamide-bisacrylamide in distills water the reaction temperature drops down to extremely. Because the reaction is energy absorbance and this could be good indicator for products. The stock solution is wrapped with aluminum foil and stored in 4 C˚. The acrylamide gel solution was allowed to polymerize for 40 minutes at the room temperature. Then used HindIII restriction enzyme and package contents Sterile, deionized water 16.3µl, RE10X Buffer 2 µl, Acetylated BSA 0.2 µl, Restriction enzyme 0.5 µl, DNA product 1 µl.

Genetic Relationships

Genetic diversity in the genome DNA, which can yield from application DNA-markers for determination genetic diversity among varieties[13]. Genetic relationships between selective cultivars which converted to characterization data to estimated similarity value by(SIMQUL) similarity for Qualitative Data, by formula Nei and Li:

Similarity = $2n\ xy / nx + ny$

Determination genetic distance between cultivars by using formula:

Genetic distance = $1- (2nxy / nx + ny) \times 100$

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Whereas nxy : Number of bands in x and y
nx : Number of all bands in x
ny: Number of all bands in y.

Results and Discussion

SSR-primer analysis

In this study used 5 microsatellites to determinate genetic diversity among sixty five date palm cultivars, profile of all of these by separated SSR-PCR products on the gel electrophoresis either one band (homozygous) or two bands (heterozygous) were revealed for each primer based on the types of microsatellite loci[14].

Table 4: summary of 5 microsatellites revealed Allele size, number of allele, number of amplified bands, and genotypes.

N.o	Primer	Allele size(bp)	N.o of Allele	N.o of Amplified bands	Genotypes
1	mpdCIR010	100 - 580	18	124	18
2	mpdCIR015	150- 200	23	48	23
3	mpdCIR016	200 - 1000	11	10	11
4	PDCAT4	280 - 500	25	80	13
5	PDCAT5	200 - 450	18	67	18

The results in this study table 4 revealed that the highest number of allele was 25 with PDCAT4 primer from 13 Genotypes and the lowest number of allele was 11 with mpdCIR016 primer from 11 Genotypes,on the other hand the highest number of amplification was 124 bands with mpdCIR010 primer whereas the lowest number of amplification was 48 bands with mpdCIR015 primer. Among the 5 SSR primer pair tested for their ability to generate expected SSR banding patterns in Iraqi date palm, these SSR primer successfully established the genotypes of the sixty five date palm cultivars. The SSR profiles revealed one or more different alleles per locus in the cultivars, with homozygous and heterozygous individuals clear identifiable. Total of 95 allele with a mean of 19 allele per locus were cored. The number of alleles per locus varied from 11(mpdCIR016) to 25 (PDCAT4). In this study SSR primer, we performed SSR genotyping method in order to estimate the genetic diversity in Iraqi date palm cultivars which are collected from Hillah city. Data revealed evidence of the useful of this technology know the number of markers appropriate for evidencing molecular polymorphisms in these cultivars. As a result, large number of SSR alleles have been shown with a mean 19 per locus and allowed to detect a relatively high degree of genetic diversity in these cultivars. Zehdi *et al*[15] reported, among the 16 SSR primer pairs for their ability to generate SSR banding patterns in Tunisian date palm, 14 have been generated of the 49 accessions genotypes. Locus mpdCIR48 did not amplify in our sampling and locus, mpdCIR44 conformed erratic amplifications as it was reported[11]. The SSR profiles revealed more than our variable alleles per locus with homozygous and heterozygous individuals. A total of 100 alleles with a mean of 7.14 alleles per locus. The number of allele per locus was differenced from 4 (mpdCIR16) to 10 (mpdCIR78). Racchi; *et al[17]*. (2013) reported, eighteen cultivars as common genotypes in Al Jurrah Oasis were analyzed by using 16 highly polymorphic microsatellite loci, a large number of SSR alleles have been revealed with a mean of 6.88 per locus that permitted detecting a relatively high degree of genetic diversity in these cultivars. And a high level of polymorphism was identified among cultivars as previously reported in date palm cultivars of Algeria, Morocco, Sudan and Tunisia by using both isoenzymes and SSR primers[18,19,20].

Genetic similarity of SSR primer

Genetic variation in this study was not only a cross of twenty four date palm cultivars, but also within each cultivar . This variation is useful to relationships between Iraqi date palm cultivars from Hillah city, however, phylogenetic analysis is more suitable for the interpretation of all possible relationships among a large group of genotypes[20]. Table 5 illustrated genetic distance with twenty four genotypes of date palm cultivars by using SSR primers.

Table 5: Genetic similarities between twenty four individuals of Iraqi date palm cultivars by SSR primers.

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	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	0	0.7693	0.2858	0.79538	0.46174	0.53455	0.67384	0.83292	0.34995	0.83292	0.89215	0.7423	0.66153	0.68509	0.88059	0.45176	0.55322	0.59668
2	0.7693	0	0.65463	0.28565	0.4689	0.3194	0.35712	0.82057	0.42853	0.93671	0.34987	0.8571	0.80529	0.75585	0.65461	0.68509	0.75585	0.39836
3	0.2858	0.65463	0	0.71424	0.47624	0.40408	0.61025	0.90346	0.28575	0.96887	0.84515	0.82065	0.72999	0.82065	0.90348	0.53455	0.71426	0.60797
4	0.79538	0.28565	0.71424	0	0.42318	0.47373	0.43447	0.74226	0.51501	0.93675	0.20209	0.83295	0.75291	0.67005	0.68507	0.55329	0.67005	0.39839
5	0.46174	0.4689	0.47624	0.42318	0	0.44666	0.32024	0.8052	0.32293	0.89334	0.46893	0.81788	0.7423	0.60793	0.80522	0.38099	0.4971	0.42857
6	0.53455	0.3194	0.40408	0.47373	0.44666	0	0.43446	0.72836	0.20202	0.80805	0.58897	0.74226	0.65116	0.68505	0.60604	0.57143	0.65459	0.37184
7	0.67384	0.35712	0.61025	0.43447	0.32024	0.43446	0	0.93943	0.43442	1.0126	0.41032	1.0227	0.93707	0.77261	0.84816	0.64286	0.6888	0.55379
8	0.83292	0.82057	0.90346	0.74226	0.8052	0.72836	0.93943	0	0.72834	0.34991	0.82062	0.51504	0.31223	0.31931	0.34987	0.57135	0.47373	0.51057
9	0.34995	0.42853	0.28575	0.51501	0.32293	0.20202	0.43442	0.72834	0	0.78239	0.62266	0.68512	0.60795	0.62263	0.67	0.45173	0.55319	0.35313
10	0.83292	0.93671	0.96887	0.93675	0.89334	0.80805	1.0126	0.34991	0.78239	0	0.97937	0.55329	0.45426	0.37793	0.40404	0.72839	0.51507	0.6079
11	0.89215	0.34987	0.84515	0.20209	0.46893	0.58897	0.41032	0.82062	0.62266	0.97937	0	0.9476	0.87807	0.69986	0.74228	0.6547	0.69986	0.49028
12	0.7423	0.8571	0.82065	0.83295	0.81788	0.74226	1.0227	0.51504	0.68512	0.55329	0.9476	0	0.27769	0.5714	0.55322	0.65465	0.67007	0.49027
13	0.66153	0.80529	0.72999	0.75291	0.7423	0.65116	0.93707	0.31223	0.60795	0.45426	0.87807	0.27769	0	0.43117	0.46896	0.49713	0.51726	0.45176
14	0.68509	0.75585	0.82065	0.67005	0.60793	0.68505	0.77261	0.31931	0.62263	0.37793	0.69986	0.5714	0.43117	0	0.4737	0.42857	0.20209	0.43117
15	0.88059	0.65461	0.90348	0.68507	0.80522	0.60604	0.84816	0.34987	0.67	0.40404	0.74228	0.55322	0.46896	0.4737	0	0.72838	0.62266	0.42315
16	0.45176	0.68509	0.53455	0.55329	0.38099	0.57143	0.64286	0.57135	0.45173	0.72839	0.6547	0.65465	0.49713	0.42857	0.72838	0	0.3194	0.4543
17	0.55322	0.75585	0.71426	0.67005	0.4971	0.65459	0.6888	0.47373	0.55319	0.51507	0.69986	0.67007	0.51726	0.20209	0.62266	0.3194	0	0.47618
18	0.59668	0.39836	0.60797	0.39839	0.42857	0.37184	0.55379	0.51057	0.35313	0.6079	0.49028	0.49027	0.45176	0.43117	0.42315	0.4543	0.47618	0
19	1.0595	0.88057	1.1339	0.75593	0.90472	0.91468	1.0326	0.37796	0.91469	0.55332	0.78246	0.60611	0.55536	0.45173	0.47373	0.74232	0.63891	0.59092
20	1.0301	0.84507	1.0879	0.8689	0.97349	0.8329	1.0618	0.53452	0.85708	0.49493	0.91468	0.47376	0.56144	0.62266	0.34991	0.90348	0.7954	0.56134
21	0.68509	0.75585	0.82065	0.67005	0.60793	0.68505	0.77261	0.31931	0.62263	0.37793	0.69986	0.5714	0.43117	0	0.4737	0.42857	0.20209	0.43117
22	0.93677	0.63882	0.9583	0.45176	0.67171	0.74224	0.7986	0.51501	0.74226	0.7423	0.4949	0.63884	0.59092	0.49485	0.55319	0.58902	0.60611	0.4312
23	0.94762	0.79533	1.0102	0.55329	0.65636	0.68059	0.82375	0.63882	0.83295	0.85715	0.55329	0.82065	0.72999	0.51509	0.78239	0.53455	0.55332	0.60797
24	1.2205	0.88059	1.2371	0.72843	0.99765	0.97931	1.0809	0.55324	1.0201	0.79538	0.75595	0.78239	0.72526	0.66998	0.58895	0.86891	0.83297	0.69655

19	20	21	22	23	24
1.0595	1.0301	0.68509	0.93677	0.94762	1.2205
0.88057	0.84507	0.75585	0.63882	0.79533	0.88059
1.1339	1.0879	0.82065	0.9583	1.0102	1.2371
0.75593	0.8689	0.67005	0.45176	0.55329	0.72843
0.90472	0.97349	0.60793	0.67171	0.65636	0.99765
0.91468	0.8329	0.68505	0.74224	0.88059	0.97931
1.0326	1.0618	0.77261	0.7986	0.82375	1.0809
0.37796	0.53452	0.31931	0.51501	0.63882	0.55324
0.91469	0.85708	0.62263	0.74226	0.83295	1.0201
0.55332	0.49493	0.37793	0.7423	0.85715	0.79538
0.78246	0.91468	0.69986	0.4949	0.55329	0.75595
0.60611	0.47376	0.5714	0.63884	0.82065	0.78239
0.55536	0.56144	0.43117	0.59092	0.72999	0.72526
0.45173	0.62266	0	0.49485	0.51509	0.66998
0.47373	0.34991	0.4737	0.55319	0.78239	0.58895
0.74232	0.90348	0.42857	0.58902	0.53455	0.86891
0.63891	0.7954	0.20209	0.60611	0.55332	0.83297
0.59092	0.56134	0.43117	0.4312	0.60797	0.69655
0	0.47379	0.45173	0.34991	0.51507	0.28565
0.47379	0	0.62266	0.62263	0.88059	0.62266
0.45173	0.62266	0	0.49485	0.51509	0.66998
0.34991	0.62263	0.49485	0	0.31944	0.34987
0.51507	0.88059	0.51509	0.31944	0	0.55322
0.28565	0.62266	0.66998	0.34987	0.55322	0

1: Breem 6: Ashrasi 11: Khestawi 16: Maddny 21: Shwethi
2: Tebarzal 7: Zahdi 12: Usta Umran 17: Barhi 22:Greatli
3: Sabb Drrah 8: Sultana 13: Guntar 18: Chipchab 23:Ghanami Ahmer
4: Hamrawi 9: Khadrawi 14: Maktom 19: Najdi 24:Smeasmi
5: Brban 10: Sukkary 15: Nersi 20: Fom Alrman

The results of matrix revealed Table 5 the similarity between date palm cultivars with SSR primers represented by the highest distance was (1.2371) between (Smeasmi) and (Sabb Drrah), that means no similarity with them cultivars, while the lowest degree of distance was (zero), which represented no distance between cultivars were, (Shwethi) and (Maktom). That means presence of similarity between them.
Zehdi *et al*. [21] reported similar with Moroccan, Algerian and Tunisian date palm cultivars. Al-Ruqiashi *et al*[22], study relationships by using SSRs and reported that Bahraini and Iraqi cultivars exhibited closely related, while Moroccan date palm were showed Futhermor, Khalas Omani (originated in Oman) and Khalas Bahraini (from Bahrain), in spite of the same name by using different molecular markers. Also, a similar distinct by the same research was that Khinaizi Oman and Khinaizi Bahraini varieties were not relationship to each other.

Phylogentic Tree

Relationships between varieties can be conducted using appropriate programs. Analyses clusters including the tested varieties are apparently related according to variation of date palm. To produced a genetic distance matrix using the formula of Nei and Li[13], which gave the similarity between any two population on the basis of the number of generated bands. The matrix was then computed with the Neighbour program based on the unweighted pair group method with the arithmetic averaging (UPGMA), Whereas computed these product treefile in the installation program (NTSYS-PC) or any Tree view program can be used to draw phylogenetic diagrams[23] . The genetic relationships among twenty four cultivars can be illustrated in the Figure 1.

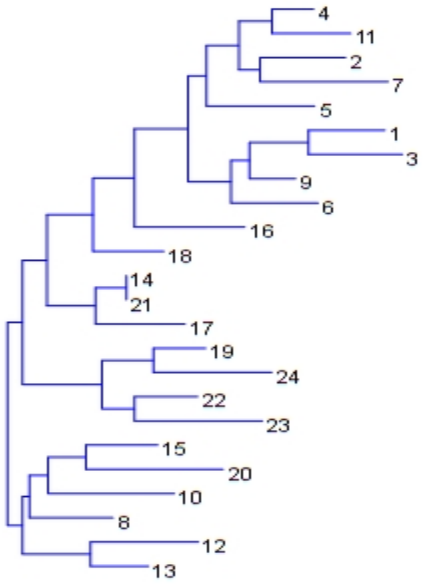

Figure 1:. Genetic relationships among twenty for date palm cultivars estimated by SSR primers from UPGMA dendrogram.
1: Breem 6: Ashrasi 11: Khestawi 16: Maddny 21: Shwethi
2: Tebarzal 7: Zahdi 12: Usta Umran 17: Barhi 22:Greatli
3: Sabb Drrah 8: Sultana 13: Guntar 18: Chipchab 23:Ghanami Ahmer
4: Hamrawi 9: Khadrawi 14: Maktom 19: Najdi 24:Smeasmi
5: Brban 10: Sukkary 15: Nersi 20: Fom Alrman
The results revealed genetic relationships among twenty four genotypes; divided in to two clusters, clusters I (untar,UstaAmran, ultana, ukkary, om alrman and nersi) and clusterII which divided into two sub groups, sub group I (Ghanami Ahmer,Greatli,Smeasmi and Najdi) and sub groups II which divided into two groups, groups A(Ghanami Ahmer, Greatli, Smeasmi andNajdi) and groups B which divided into two groups, there are groups A′(Barhi, Shwethi and

maktom) and groups B′(Chipchab, Maddany, Ashrasi, Khadrawi, SabbDrrah,Breem, Barban, Zahdi, Tebarzal,Khestawi and Hamrawi). Trifi, *et al*.[23] reported genetic relationships among the seventy tested primers; only thirty one were used to assess relationships. Smouse and Peakall[24] nestimating the average dissimilarity internal to each cultivars. In seven varieties from eighteen absences of genetic diversity within variety was observed in agreement with results. Similar result was shown by Zehdi *et al[16]*. in the analysis of 49 Tunisian date palm with three SSR loci.

ITS-primer analysis

This study used universal primers ITS F and ITS R, the amplification of ITS using the PCR cycling parameters by [25]cross twenty four date palm cultivars in molecular size nearby 80bp. The PCR products as individual bands of ITS region were reveled on the 2% agarose gel electrophoresis.

PCR-RFLP analysis

The purified PCR products were subjected to single digestion with HindIII enzyme, overnight at 37C˚ in 20µl reaction volume. he digested fragments were resolved an acrylamide gel. The analysis was performed to reveal varieties fingerprinting with the Sultana, Sukkary and Maktom cultivars that mean number of fingerprinting was 3. The results of PCR-RFLP in this study are summarized in Table 6. There were 5 estimated positions for main bands across the cultivars which used in this study.

Table 6 : PCR- RFLP with restriction enzyme Hind III: molecular size of bands, their presence (+), or their absence (-), no. of polymorphic bands, no. of defined varieties, similarity and total of bands.
Lane A: Breem Lane F: Ashrasi LaneK: Khestawi Lane P: Maddny Lane U: Shwethi
Lane B: Tebarzal Lane G: Zahdi Lane L: Usta Umran Lane Q: Barhi Lane V:Greatli
Lane C: Sabb Drrah Lane H: Sultana Lane M: Guntar Lane R: Chipchab Lane W:Ghanami Ahmer
Lane D: Hamrawi Lane I: Khadrawi Lane N: Maktom Lane S: Najdi LaneX:Smeasmi
Lane E: Brban Lane J: Sukkary Lane O: Nersi Lane T: Fom Alrman

M.S bp	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
80	_	_	_	_	_	_	_	_	+	_	+	+	+	+	_	+	+	+	+	+	+	+	+	+
75	_	_	_	_	_	_	_	+	_	+	_	_	_	_	+	_	_	_	_	_	_	_	_	_
65	+	+	+	+	+	+	+	_	_	_	_	+	+	+	+	+	+	+	+	+	_	_	_	_
55	_	_	_	_	_	_	_	_	+	+	+	_	_	_	_	_	_	_	_	_	+	+	+	+
50	+	+	+	+	+	+	+	+	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
Total	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2

POL
Total bands= 48
NO. of defined varieties
Similarity

The results of PCR- RFLP revealed in the Table 6, five estimated position have been used for main bands across 24 date palm cultivars ranging in their molecular size from nearby 50 bp to 80 bp. There were 5 numbers of defined varieties; the polymorphic products were 6 bands representing Sultans, Sukkary and Nersi, the percentage of similarity was 87.5% with the Breem, Tebarzal, SabbDrrah, Hamrawi, Barban, Ashrasi, Zahdi, Khadrawi, Khestawi, UstaUmran, Gunar, Maktom, Maddny, Barhi, Chipchab, Najdi, Fom Alrman, Shwethi, Greatli, Ganami Ahmer and Smeasmi, and the total of bands were 48 bands. Abass[26] was reported a PCR ITS-RFLP technique for identifying fungal contamination of date palm (*phoenix dactylifera* L.) tissue cultures. The restriction fragment length polymorphism (RFLP) of ITS amplicons was used to discriminate between fungal species and provides an alternative method to sequencing ITS products, both restriction endonucleases *EcoR*1 and *Smal* were used to digest ITS products. Finally the ITS-RFLP technique regarded efficiency for identified the varieties of date palm, cost and needed amount of DNA.

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